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(54) **Title:** COMPOSITION FOR TREATMENT OF AUTOIMMUNE DISEASE

(57) **Abstract:** The present invention provides novel compositions and methods for inhibiting immune responses associated with autoimmune diseases and allergic responses. In particular, it relates to vaccination with compositions comprising an adjuvant comprising cell wall skeleton ("CWS") from a *Mycobacterium* sp. and peptides from the hypervariable region of MHC molecules encoded by alleles associated with disease.

WO 01/74375 A1

Composition for Treatment of Autoimmune Disease

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims priority to USSN 60/194,547, filed April 4, 2000, and USSN 60/247,117, filed November 10, 2000, each of which is incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

A number of pathological responses involving unwanted immune responses are known. For instance, a number of allergic diseases, have been associated with particular MHC alleles or suspected of having an autoimmune component. Other deleterious T cell-mediated responses include the destruction of foreign cells that are purposely introduced into the body as grafts or transplants from allogeneic hosts. This process, known as "allograft rejection," involves the interaction of host T cells with foreign MHC molecules. Quite often, a broad range of MHC alleles are involved in the response of the host to an allograft.

Autoimmune disease is a particularly important class of deleterious immune response. In autoimmune diseases, self-tolerance is lost and the immune system attacks "self" tissue as if it were a foreign target. More than 30 autoimmune diseases are presently known; these include many which have received much public attention, including rheumatoid arthritis (RA), myasthenia gravis (MG), and multiple sclerosis (MS).

A crude approach to treating autoimmune disease and other immunopathologies is general immunosuppression. This approach has the obvious disadvantage of crippling the ability of the subject to respond to real foreign materials to which it needs to mount an immune response. Recent approaches to treating autoimmune disease have involved the use of peptides having an amino acid sequence encoded by a T-cell receptor V gene. The peptides, or DNA encoding the peptides, have been proposed as

vaccines for preventing, suppressing and treating immune related diseases (*see* International Publication No. WO 91/01133). Another approach involves the use of monoclonal antibodies against MHC gene products. The antibodies have been proposed for use in targeting cells bearing MHC molecules associated with particular diseases (*see* EP Publication No. 68790).

These prior art methods fail, however, to provide a simple self-mediated method for specifically eliminating immune responses restricted by glycoproteins encoded by MHC alleles associated with a variety of deleterious immune responses. Such methods can be used to prevent and/or suppress diseases, particularly those in which the antigen or allergen is not known.

SUMMARY OF THE INVENTION

The present invention thus provides novel compositions and methods for inhibiting immune responses associated with autoimmune diseases and allergic responses. In one aspect, it relates to the vaccination with compositions comprising an adjuvant comprising cell wall skeleton ("CWS") from a *Mycobacterium* species, and peptides from the hypervariable region of MHC class II molecules encoded by alleles associated with the particular disease of interest.

In one aspect, the present invention provides a pharmaceutical composition comprising an isolated immunogenic MHC class II polypeptide, wherein the polypeptide has an amino acid sequence from a hypervariable region of an MHC class II molecule, in combination with an adjuvant comprising isolated cell wall skeleton from a *Mycobacterium* species.

In one embodiment, the *Mycobacterium* is *Mycobacterium phlei*.

In one embodiment, the composition further comprises an oil-in-water emulsion. In another embodiment, the oil is squalane oil. In another embodiment, the adjuvant comprises a detergent. In another embodiment, the detergent is Tween-80. In another embodiment, the adjuvant comprises an oil-in-water emulsion comprising squalene oil and Tween-80.

In one aspect, the MHC class II molecule is from a human. In another aspect, the MHC class II molecule is from a mouse. In yet another aspect, the polypeptide is about 15 to about 25 amino acids in length.

In some embodiments, the MHC class II molecule is an MHC class II β chain. In one embodiment, the MHC class II molecule is a human HLA-DR β chain. In one

embodiment, the MHC class II molecule is a human HLA-DR4 β chain. In another embodiment, the MHC class II molecule is encoded by the HLA-DR B1*0401 allele or the HLA-DR B1*0101 allele. In another embodiment, the polypeptide comprises an amino acid sequence corresponding to amino acids 57-76 of a human HLA-DR β chain. In another embodiment, the polypeptide comprises the amino acid sequence DAEYWNSQKDLLEQKRAAVD or DAEYWNSQKDLLEQRRAAVD. In another embodiment, the polypeptide further comprises a cysteine amino acid residue at the N-terminus.

In another embodiment, the MHC class II molecule is a peptide that corresponds to the susceptibility region of the human HLA-DQ allele. In one embodiment, the MHC class II molecule is encoded by the HLA-DQ B1*0401 allele, the HLA-DQ B1*0303(2) allele, the HLA-DQ B1*0201 allele or the HLA-DQ B1*0302 allele. In one embodiment, the polypeptide comprises an amino acid sequence corresponding to amino acids 43-62 of the human HLA-DQ β chain. In another embodiment, the polypeptide comprises the amino acid sequence DVGEYRAVTELGRHSAEYYN, DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, or DVGVYRAVTPLGPPAAEYWN. In another embodiment, the polypeptide comprises an amino acid sequence corresponding to amino acids 54-76 of the human HLA-DQ β chain. In another embodiment, the polypeptide comprises the amino acid sequence GRHSAEYYNKQYLERTRAELDTA, GRLDAEYWNSQKDILEEDRASVD, GPPDAEYWNSQKEVLERTRAELD, GLPAAEYWNSQKDILERKRAAVD, or GPPAAEYWNSQKEVLERTRAELD.

In other embodiments, the polypeptide has an acetylated N-terminal amino acid.

In one embodiment, the polypeptide has an amino acid sequence from an MHC class II molecule encoded by an allele associated with an autoimmune disease. In another embodiment, the autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

In another aspect, the present invention provides a pharmaceutical composition comprising an isolated immunogenic MHC class II polypeptide, wherein the polypeptide has the amino acid sequence DAEYWNSQKDLLEQKRAAVD, DAEYWNSQKDLLEQRRAAVD, DVGEYRAVTELGRHSAEYYN,

DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, DVGVYRAVTPLGPPAAEYWN, GRHSAEYYNKQYLERTRAELDTA, GRLDAEYWNSQKDILEEDRASVD, GPPDAEYWNSQKEVLERTRAELD, GLPAAEYWNSQKDILERKRAAVD, or GPPAAEYWNSQKEVLERTRAELD in combination with an adjuvant comprising an immunostimulatory compound, wherein the immunostimulatory compound is isolated cell wall skeleton from *Mycobacterium phlei*.

In one embodiment, the adjuvant further comprises Tween-80. In another embodiment, the adjuvant further comprises Tween-80 and squalene oil.

In another aspect, the present invention provides a method of eliciting an immune response in a subject, the method comprising the step of administering to the subject an immunologically effective amount of a pharmaceutical composition comprising an isolated immunogenic MHC class II polypeptide, wherein the polypeptide has an amino acid sequence from a hypervariable region of an MHC class II molecule, in combination with an adjuvant comprising isolated cell wall skeleton from a *Mycobacterium* species.

In one embodiment, the subject is a human.

In one embodiment, the composition is administered intramuscularly. In one embodiment, the composition is administered prophylactically. In another embodiment, the composition is administered to a subject having an autoimmune disease such as, *e.g.*, rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

In another embodiment, the composition is administered in an immunologically effective amount of about 1 mg polypeptide to about 15 mg polypeptide, or 0.1 mg to 10 mg or polypeptide.

In another aspect, the present invention provides a pharmaceutical composition comprising a MHC class II molecule corresponding to amino acids 43-62 of the human HLA-DQ β chain, and a pharmaceutically acceptable carrier. In one embodiment, the pharmaceutical composition comprises an adjuvant. In an additional aspect, the present invention provides a method of eliciting an immune response in a subject, the method comprising the step of administering to the subject an immunologically effective amount of the above-described pharmaceutical composition. In one embodiment, the composition is administered intramuscularly. In another embodiment, the composition is administered prophylactically. The composition may be administered to a subject having an autoimmune

disease such as, *e.g.*, rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a list of the DQ/DR haplotypes in humans and their associations with autoimmune disease.

Figure 2 provides a schematic diagram of an idealized CWS, based on the CWS of *M. tuberculosis*.

Figure 3 provides a diagram of a process for making bulk raw CWS.

Figure 4 provides a diagram of a manufacturing process for CWS.

Figure 5 (A-E) shows disease progression in an EAE mouse model, for mice that were (A) untreated; (B) treated with CWS alone; (C) treated with *M. phlei* CWS plus polypeptide; (D) treated with CFA plus polypeptide, and (E) treated with CFA alone.

Figure 6 provides a summary of EAE incidence in the control and vaccinated mice.

Figure 7 shows mouse and human amino acid sequences corresponding to amino acids 57-76 of the MHC class II DR β chain (SEQ ID NOS:1-2); amino acids 43-62 of the MHC class II DQ β chain (SEQ ID NOS:3-7); and amino acids 54-76 of the MHC class II DQ β chain (SEQ ID NOS:8-12).

Figure 8 shows the results of an experiments in which CWS-AQ plus the peptides of the invention reduced the incidence of EAE in SJL mice.

Figure 9 shows the results of an experiments in which CWS-AQ plus the peptides of the invention reduced the incidence of EAE in SJL mice.

Figure 10 shows a summary of the effects of adjuvants plus the peptides of the invention in EAE mice.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

I. INTRODUCTION

The present invention provides polypeptide and polypeptide-adjuvant compositions for treatment and prophylaxis of allergies and autoimmune diseases. The compositions may include an adjuvant comprising cell wall skeleton ("CWS") from a *Mycobacterium* species, preferably from *M. phlei*. The compositions optionally include

additives such as oil, preferably squalane oil; and/or a detergent/surfactant, preferably Tween-80 (polyoxyethelene (20) sorbitan monooleate, *see, e.g.*, *The Merk Index*, p. 1308 (Budavari *et al.*, eds., 12^{th} ed.), as well as other pharmaceutically acceptable components. The polypeptides of the invention correspond to a hypervariable region from an MHC class II molecule associated with a disease state (*see* Figure 1; *see also* WO 96/40230 and WO 94/13320 for a description of the polypeptides of the invention). The compositions of the invention can be used for prophylaxis and treatment of allergies, such as ragweed allergy, and of autoimmune diseases, such as diabetes, myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.

The polypeptides or adjuvant-polypeptide compositions are delivered to the subject in a pharmaceutically acceptable carrier such as an aqueous solution (*e.g.*, water, saline), oil-in-water emulsion, precipitated aluminum salts, or liposomes. In one embodiment, the composition comprises an oil-in-water emulsion, using any suitable metabolizable oil, such as mineral oils, fatty oils of vegetable or animal origin, fatty esters, or polyols, preferably mineral oil, squalene oil, squalane oil, *etc.* Preferably, squalane oil is used. Often, the CWS is first dissolved in the oil and then dispersed in the water phase. Typically, the water phase is water for injection (*e.g.*, water purified by distillation or reverse osmosis) or a pharmaceutically acceptable aqueous solution such as saline, *e.g.*, PBS, with antigen dissolved therein. The amount of oil in the final oil-in-water emulsion is typically about 0.1% to 10% oil, preferably about 1-2% oil. The adjuvant composition can also contain additives, such as a detergent or surfactant, *e.g.*, sorbitan fatty acid esters and their derivatives such as Tween, preferably Tween-80. The amount of detergent is typically about 0.01% to about 1%, typically about 0.1-0.2%. The amount of antigen (MHC class II hypervariable region polypeptide) is typically incorporated into the composition at a concentration of about 0.001 mg/ml to about 10 mg/ml, preferably about 0.05 mg/ml to 0.25 mg/ml. The adjuvant composition or the adjuvant-polypeptide composition can also contain other additives, such as antioxidants and preservatives.

II. DEFINITIONS

As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

An "adjuvant" refers to the components in a vaccine or therapeutic composition that increase the specific immune response to the antigen of interest (*see, e.g.*, Edelman, *AIDS Res. Hum. Retroviruses* 8:1409-1411 (1992)). Adjuvants may induce

immune responses of the Th1-type and Th-2 type response. Th1-type cytokines (*e.g.*, IFN-γ, IL-2, and IL-12) tend to favor the induction of cell-mediated immune response to an administered antigen, while Th-2 type cytokines (*e.g.*, IL-4, IL-5, IL-6, IL-10 and TNF-β) tend to favor the induction of humoral immune responses.

"Cell wall skeleton" or "CWS" refers to cell wall skeleton from a *Mycobacterium* species, preferably *M. phlei*. CWS from *Mycobacterium* sp. is a complex biological polymer consisting of the insoluble matrix of the mycobacterial cell wall after removal of soluble proteins, lipids, carbohydrates and nucleic acids by solvent extractions and enzymatic treatment with proteases and nucleases. These large heteropolymers contain three covalently attached macromolecules, peptidoglycan (PG), arabinogalactan (AG) and mycolic acids. Unextractable protein is associated with the peptidoglycan in an unknown manner. Figure 1 shows a schematic representation of an idealized *Mycobacterium* CWS. CWS can be made according to the methods described herein, and can also be made according to methods known to those of skill in the art (*see*, *e.g.*, U.S. Patent No. 4,436,727). "CWS-AQ" refers to an aqueous solution comprising CWS with polysorbate 80 (Tween 80). "CWS-SE" refers to an aqueous solution comprising squalene oil, CWS, and polysorbate 80 (Tween 80).

An "immunogenic" MHC class II polypeptide of the present invention is a polypeptide capable of eliciting an immune response against an MHC class II molecule associated with a deleterious immune response in a patient. As set forth in more detail below, the sequence of residues in the polypeptide will be identical to or substantially identical to a polypeptide sequence from the hypervariable region of the MHC class II molecule of interest. Thus, a polypeptide of the invention that has a sequence "from a region in an MHC molecule" (*e.g.*, the hypervariable region) is a polypeptide that has a sequence either identical to or substantially identical to the naturally occurring MHC amino acid sequence of the region, *e.g.*, a conservatively modified variant. As described below, the polypeptide can comprise amino acid analogs and mimetics.

As used herein a "hypervariable region" of an MHC class II molecule is a region of the molecule in which polypeptides encoded by different alleles at the same locus have high sequence variability or polymorphism. The polymorphism is typically concentrated in the α1 and β1 domains of Class II molecules. The number of alleles and degree of polymorphism among alleles may vary at different loci. For instance, in HLA-DR molecules all the polymorphism is attributed to the β chain and the α chain is relatively

invariant. For HLA-DQ, both the α and β chains are polymorphic. The hypervariable regions can be determined by methods known to those of skill in the art, *e.g.*, using a sequence comparison algorithm as described below (*see also* Brown *et al.*, *Nature* 364:33-39 (1993)). For example, the MHC class II DR β chain has a region of hypervariability from amino acids 57-76, while the MHC class II DQ β chain has a region of hypervariability from amino acids 43-62 and 54-76 (*see, e.g.,* SEQ ID NOS:1-12).

"Vaccine" as used herein refers to a composition comprising an immunogenic polypeptide, or a DNA molecule encoding an immunogenic polypeptide, wherein the polypeptide elicits an antibody and/or a cell-mediated immune response in a subject.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the MHC class II polypeptides of this invention do not contain materials normally associated with their *in situ* environment, *e.g.*, other surface proteins on antigen presenting cells. Even where a protein has been isolated to a homogenous or dominant band, there are trace contaminants in the range of 5-10% of native protein which co-purify with the desired protein. Isolated polypeptides of this invention do not contain such endogenous co-purified protein.

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The

term nucleic acid is used interchangeably herein with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. The term "amino acid analogs" refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, or methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. The term "amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified

variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

(*see, e.g.*, Creighton, *Proteins* (1984)).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acids, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is

the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C depending on primer length. For high stringency PCR amplification, a temperature of about 62°C is typical, although high stringency annealing temperatures can range from about 50°C to about 65°C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90°C - 95°C for 30 sec - 2 min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72°C for 1 - 2 min.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the

same (*i.e.*, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more identity over a specified region, *e.g.*, a hypervariable region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the complement of a test sequence. Preferably, the identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. For sequence comparison of nucleic acids and proteins to MHC class II hypervariable regions and nucleic acids encoding them, the BLAST and BLAST 2.0 algorithms and the default parameters discussed below are used.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g.*, Ausubel *et al.* (eds), *Current Protocols in Molecular Biology* (1995 supplement)).

A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which

are described in Altschul *et al.*, *Nuc. Acids Res.* 25:3389-3402 (1977), and Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Natl. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

"Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990)).

For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985)). Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used

to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al., Nature* 348:552-554 (1990); Marks *et al., Biotechnology* 10:779-783 (1992)).

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

The term "immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein, e.g., a specific family of proteins, or a specific ortholog, allele, etc. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

III. CWS ADJUVANTS

The present invention provides an adjuvant composition for administering a polypeptide from the hypervariable region of an MHC class II molecule. The adjuvant composition comprises CWS from a *Mycobacterium* species (*see* U.S. Patent No. 4,436,727). CWS from *Mycobacterium* sp. is a complex biological polymer consisting of the insoluble

matrix of the mycobacterial cell wall after removal of soluble proteins, lipids, carbohydrates and nucleic acids by solvent extractions and enzymatic treatment with proteases and nucleases. These large heteropolymers contain three covalently attached macromolecules, peptidoglycan (PG), arabinogalactan (AG) and mycolic acids. Unextractable protein is associated with the peptidoglycan in an unknown manner. While details of the absolute structural configuration for *M. phlei* CWS are not known, the structures of CWS from other mycobacterial species have been investigated (reviewed by Brennan & Nikaido, *Annu. Rev. Biochem.* 64:29-63 (1995)). An idealized CWS structure based on the CWS of *M. tuberculosis* is shown in Figure 2. A method for making CWS from a *Mycobacterium* species is described below. Although this method is used for making CWS from *M. phlei*, it can be used to make CWS from other *Mycobacterium* species. In addition, other methods of making CWS are know to those of skill in the art (*see, e.g.*, U.S. Patent No. 4,436,727).

The *Mycobacterium* species can be any suitable mycobacterium, including, *e.g.*, *M. tuberculosis*, *M. bovis*, *M. smegmatis*, *BCG*, *M. leprae*, *M. scrofulaceum*, *M. avium-intracellulare*, *M. marinum*, *M. ulcerans*, *M. kansasii*, *M. xenopi*, *M. szulgai*, *M. fortuium*, *M. chelonae*, *M. africanum*, *M. abscessus*, *M. celatum*, *M. genavense*, *M. gordonae*, *M. haemophilum*, *M. malmoense*, *M. simiae*, *M. vaccae*, *etc.*

In a preferred embodiment, CWS of *Mycobacterium phlei* is used in the adjuvant composition of the present invention. *M. phlei* CWS is manufactured by Corixa. For production of *M. phlei* CWS, the original *M. phlei* culture, strain 110, was obtained from the Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT. Master and working cell banks were prepared at Corixa and characterized by standard bacteriological tests and fatty acid profile. *M. phlei* cultures are grown as a pellicle in plastic culture vessels at 35° to 37°C using a defined media. Cultures are harvested using nylon filters, and the cells are disrupted using the Sorvall-Ribi Cell Fractionator.

Bulk CWS is produced by sequential nuclease and protease digestion of the cell walls followed by aqueous and alcohol washes using centrifugation. CWS can also be purchased from Corixa Corporation, Hamilton, Montana (catalogue numbers 250 and 251). Final purification is by organic solvent extraction followed by further alcohol and aqueous washes. CWS is terminally sterilized by autoclaving at 121°C for 25 minutes and subsequently lyophilized. CWS is stored at 2° to 8°C prior to reconstitution with solution and antigen. An overview of cell growth and bulk CWS production is provided in Figure 3. Production of bulk CWS is performed in a dedicated suite using dedicated CWS equipment. The suite contains a pass-through autoclave; all materials are sterilized prior to leaving the

The concentrated emulsion is transferred into the depyrogenated, sterile 4 liter jacketed stainless steel blending vessel. A fourteen-inch shearing head attached to a Silverson Homogenizer is lowered into the blending vessel and the vessel is sealed. Water is circulated through the jacket of the vessel to control the vessel temperature to 70° ± 5°C. The concentrated emulsion is blended at 10,800 ± 300 RPM while the remainder of the sterile polysorbate 80 aqueous phase solution is added at a rate of 64 to 80 ml/minute. After all the sterile polysorbate 80 aqueous phase solution has been added, the mixing speed is reduced to 2000 RPM and the jacket temperature is reduced to 2°-8°C. The mixing can be stopped at this point, and the emulsion transferred to a 4 liter screw-top flask and stored overnight at 2°-8°C if required.

The 4 liter flask is stirred on a stir plate at a rate sufficient to create a vortex. The emulsion is continually mixed at 8,000 RPM using the Silverson Homogenizer, and two 10 ml samples are removed and submitted to QC for bulk sterility testing. The 4 liter flask is connected to the Filamatic automated fill machine, and 100 µl of stable emulsion are dispensed into each 3 ml vial. After filling, stoppers are fully inserted into the vials, each vial is fitted with an aluminum seal, and the seal is crimped in place. The vials are transferred into a quarantine storage location and held at 2°-8°C pending QC release testing and QA lot release.

A preferred stock CWS-SE solution is a 3 ml stock solution of *M. phlei* CWS adjuvant (Corixa Corporation, Hamilton, Montana) containing 1500 µg/ml CWS, 81.15 mg/ml squalane oil, and 20 mg/ml surfactant such as Tween-80. A preferred stock CWS-AQ solution is a 3 ml stock solution of *M. phlei* CWS adjuvant (Corixa Corporation, Hamilton, Montana) containing 1000 µg/ml CWS, 0.03% (V/V) surfactant such as Tween-80, and 0.9% saline (W/V). For administration to a mouse, a 1:10 dilution of CWS stock (SE or AQ) and a 1:10 dilution of peptide in PBS is prepared (stock peptide is at 10 mg/ml, use 200 µg/mouse). 200 µl of this preparation are injected per mouse. For 10 mice, a total of 2 ml of preparation are needed, containing: 0.2 ml of stock peptide, 0.2 ml CWS stock, and 1.6 ml PBS. For administration to a larger mammalian subject, such as a human, the dose is scaled accordingly.

IV. MHC CLASS II POLYPEPTIDES FROM HYPERVARIABLE REGIONS

A. MHC Class II molecules

The present invention provides immunogenic polypeptides derived from the Major Histocompatibility Complex (MHC) class II glycoprotein protein sequences for use in compositions and methods for the treatment, prevention and diagnosis of deleterious immune responses. The polypeptides are capable of inducing an immune response against glycoproteins encoded by MHC class II alleles associated with the target disease. The polypeptides of the invention are derived from hypervariable regions of the α or β chain of an MHC Class II molecule associated with the deleterious immune response. In this way, the ability of antigen presenting cells (APC) to present the target antigen (*e.g.*, autoantigen or allergen) is inhibited.

The glycoproteins encoded by the MHC have been extensively studied in both the human and murine systems. Many of the histocompatibility proteins have been isolated and characterized. For a general review of MHC glycoprotein structure and function, *see, e.g., Fundamental Immunology* (3d ed., Paul, ed., 1993).

MHC molecules are heterodimeric glycoproteins expressed on cells of higher vertebrates and play a role in immune responses. In humans, these molecules are referred to as human leukocyte antigens (HLA). MHC glycoproteins are divided into two groups, class I and class II, which differ structurally and functionally from each other. In general, the major function of MHC molecules is to bind antigenic peptides and display them on the surface of cells. Class II MHC molecules are expressed primarily on cells involved in initiating and sustaining immune responses, such as T lymphocytes, B lymphocytes, macrophages, and the like. Class II MHC molecules are recognized by helper T lymphocytes and induce proliferation of helper T lymphocytes and amplification of the immune response to the particular antigenic peptide that is displayed.

Engagement of the T cell receptor induces a series of molecular events characteristic of cell activation, such as, increase in tyrosine phosphorylation, Ca^{++} influx, PI turnover, synthesis of cytokines and cytokine receptors, and cell division (*see*, *e.g.*, Altman *et al.*, *Adv. Immunol.* 48:227-360 (1990)). For a general discussion of how T cells recognize antigens *see*, Grey *et al.*, *Scientific American*, pp. 56-64 (November, 1989).

In mice, MHC class II molecules are encoded by the I-A and I-E subregions. The isolated antigens encoded by the murine I-A and I-E subregions have been shown to consist of two noncovalently bonded peptide chains: an α chain of 32-38 kD and a β chain of 26-29 kD. A third, invariant, 31 kD peptide is noncovalently associated with these two

peptides, but it is not polymorphic and does not appear to be a component of the antigens on the cell surface. The α and β chains of a number of allelic variants of the I-A region have been cloned and sequenced.

Human Class II (encoded by alleles at the HLA-DR, -DP, and -DQ loci) glycoproteins have a domain structure, including antigen binding sites, similar to that of Class I. The Class II molecules comprise two chains, the α and β chains, which extend from the membrane bilayer. Each subunit in Class II molecules consists of globular domains, referred to as α1, α2, β1, and β2. All except the α1 domain are stabilized by intrachain disulfide bonds typical of molecules in the immunoglobulin superfamily. The N-terminal portions of the α and β chains, the α1 and β1 domains, contain hypervariable regions which are thought to comprise the majority of the antigen-binding sites (*see*, Brown *et al.*, *Nature* 364:33-39 (1993)).

As noted above, each MHC allele encodes proteins which comprise hypervariable regions and antigen binding sites specific for particular sets of antigenic peptides. If the peptides bound by the MHC molecule are from an autoantigen, allergen or other protein associated with a deleterious immune response, the hypervariable region of the MHC molecule can be used to produce immunogenic polypeptides which will elicit an immune response against the MHC molecule (*see, e.g.*, Topham, *Proc. Natl. Acad. Sci. USA* 91:8005-8009 (1994); Bright *et al.*, *J. Neuroimmunol.* 67:119-124 (1996)) These polypeptides are, therefore, useful in targeting particular gene products associated with deleterious immune responses because the immune response against the MHC molecule will inhibit antigen presentation associated with the deleterious immune response. The polypeptides of the invention are typically at least about 9 or 10 amino acids in length, often from about 15, 20, or 25 amino acids in length, often from 10 to 25 amino acids in length.

In one embodiment, the polypeptide corresponds to amino acids 57-76 of the of the third hypervariable region of the beta chain encoded by the DR MHC class II allele. In one embodiment, the MHC class II polypeptide of the invention has the amino acid sequence DAEYWNSQKDLLEQKRAAVD set forth in SEQ ID NO:1 (encoded by the HLA-DR-B1*0401 allele). In another embodiment, the MHC class II polypeptide of the invention has the amino acid sequence DAEYWNSQKDLLEQRRAAVD set forth in SEQ ID NO:2 (encoded by the HLA-DR-B1*0101 allele). Such polypeptides are useful for treating and diagnosing, e.g., rheumatoid arthritis and other autoimmune diseases.

In another embodiment, the polypeptide corresponds to amino acids 43-62 of the third hypervariable region of the beta chain of the human DQ MHC class II molecule, and

overlaps with the amino acid positions that define the susceptibility regions of the human DQ allele. In another embodiment, the polypeptide is a mouse polypeptide corresponding to amino acids 43-62 of the third hypervariable region of the beta chain of the MHC class II molecule. In one embodiment, the mouse polypeptide has the sequence DVGEYRAVTELGRHSAEYYN set forth in SEQ ID NO:3. Human polypeptides corresponding to amino acids 43-62 of the human DQ allele are: DVGVYRAVTPLGRLDAEYWN (SEQ ID NO:4), DVGVYRAVTPLGPPDAEYWN (SEQ ID NO:5), DVGEFRAVTLLGLPAAEYWN (SEQ ID NO:6), and DVGVYRAVTPLGPPAAEYWN (SEQ ID NO:7). Such polypeptides are useful for diagnosing and treating, e.g., diabetes and other autoimmune diseases.

In another embodiment, the polypeptide corresponds to amino acids 54-76 of the third hypervariable region of the beta chain of the DQ MHC class II molecule and defines a Class II region in which there is a substantial T cell proliferative response in naïve NOD mice (*see, e.g.*, Chaturvedi *et al., J. Immunol.* 164:6610-6620 (2000)). In still another embodiment, the mouse MHC class II polypeptide has the amino acid sequence GRHSAEYYNKQYLERTRAELDTA set forth in SEQ ID NO:8. Human polypeptides corresponding to amino acids 54-76 of the human DQ allele are: GRLDAEYWNSQKDILEEDRASVD (SEQ ID NO:9), GPPDAEYWNSQKEVLERTRAELD (SEQ ID NO:10), GLPAAEYWNSQKDILERKRAAVD (SEQ ID NO:11), and GPPAAEYWNSQKEVLERTRAELD (SEQ ID NO:12). Such polypeptides are useful for diagnosing and treating, e.g., diabetes and other autoimmune diseases.

In one embodiment, the polypeptide comprises at least amino acids 54-62 of the third hypervariable region of the beta chain of the DQ MHC class II molecule. Polypeptides comprising amino acids 54-62 of this region often comprise up to about 25 amino acids in length.

Thus, immunization with the polypeptides will be haplotype specific and result only in the inhibition of the immune response mediated by the target molecules, while leaving other alleles unaffected. Most individuals are heterozygous at each MHC locus, *e.g.*, the HLA-DR or the HLA-DQ locus. Therefore, haplotype specific therapy of disease by immunization with polypeptides of the disease susceptibility gene products of MHC genes offers a novel means of immunotherapy. This therapy is unlikely to bring about global immunosuppression since other alleles at the particular locus will be unaffected.

B. How to make the polypeptides of the invention

Polypeptides suitable for use in the present invention can be obtained in a variety of ways. Conveniently, they can be synthesized by conventional techniques employing automatic synthesizers, such as the Beckman, Applied Biosystems, or other commonly available peptide synthesizers using well known protocols. They can also be synthesized manually using techniques well known in the art (*see, e.g.*, Stewart and Young, *Solid Phase Peptide Synthesis* (2d ed. 1984)).

Alternatively, DNA sequences which encode the particular MHC polypeptide of interest may be cloned and expressed to provide the peptide. Cells comprising a variety of MHC genes are readily available and may, for instance, be obtained from the American Type Culture Collection ("*Catalogue of Cell Lines and Hybridomas*," 6th edition (1988)). Standard techniques can be used to screen cDNA libraries to identify sequences encoding the desired sequences (*see*, Sambrook *et al.*, *Molecular Cloning - A Laboratory Manual* (1989); Ausubel *et al.*, *supra*). Fusion proteins (those consisting of all or part of the amino acid sequences of two or more proteins) can be recombinantly produced. In addition, using *in vitro* mutagenesis techniques, unrelated proteins can be mutated to comprise the appropriate sequences.

MHC class II glycoproteins from a variety of natural sources are also conveniently isolated using standard protein purification techniques. Peptides can be purified by any of a variety of known techniques, including, for example, reverse phase high-performance liquid chromatography (HPLC), ion-exchange or immunoaffinity chromatography, separation be size, or electrophoresis (*see generally*, Scopes, *Protein Purification* (1982)).

It will be understood that the immunogenic MHC class II polypeptides of the present invention may be modified to provide a variety of desired attributes, *e.g.*, improved pharmacological characteristics, while increasing or at least retaining substantially all of the biological activity of the unmodified peptide. For instance, the peptides can be modified by extending or decreasing the amino acid sequence of the peptide. Substitutions with different amino acids or amino acid mimetics can also be made. For example, a cysteine residue can be added at the N-terminus of a polypeptide.

As noted above, the peptides employed in the subject invention need not be identical, but may be substantially identical, to the corresponding sequence of the target MHC class II molecule. The peptides may, therefore, be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such

changes might provide for certain advantages in their use. The polypeptides of the invention can be modified in a number of ways so long as they comprise a sequence substantially identical (as defined below) to a sequence in the target region of the MHC class II molecule.

The polypeptides of the invention typically comprise at least about 9 or 10 residues and more preferably at least about 15 residues. In certain embodiments the peptides will not exceed about 50 residues and typically will not exceed about 20-25 residues. In other embodiments, the entire subunit (α or β chain) or large portions of the molecules are used. For instance, the polypeptides can comprise an extracellular domain from an MHC subunit (about 90-100 residues). Typically, the N-terminal domain (β1 or α1) is used. The entire extracellular region (*e.g.*, β1 and β2 or α1 and α2 of class II molecules) from the subunit can also be used. Thus, a wide range of polypeptide sizes may be used in the present invention.

C. How to select the MHC II polypeptides of the invention

In order to select the MHC molecules for producing peptides of the invention, particular MHC molecules that are involved in the presentation of the antigen of interest are identified.

In the case of allergies, specific allergic responses are correlated with specific MHC types. For instance, allergic reactions to ragweed are known to be associated with DR2 alleles (*see, e.g.*, Marsh *et al.*, *Cold Spring Harb. Symp. Quant. Biol.* 54:459-70 (1989)).

Specific autoimmune dysfunctions are also correlated with specific MHC types. A list of the DQ/DR haplotypes in humans and their associations with autoimmune diseases is shown in Figure 1. Methods for identifying which alleles, and subsequently which MHC encoded polypeptides, are associated with an autoimmune disease are known in the art. Suitable methods are described, for instance, in EP publication No. 286447. In this method several steps are followed.

First, the association between an MHC antigen and the autoimmune disease is determined based upon genetic studies. The methods for carrying out these studies are known to those skilled in the art, and information on all known HLA disease associations in humans is maintained in the HLA and Disease Registry in Copenhagen. The locus encoding the polypeptide associated with the disease is the one that would bear the strongest association with the disease.

Second, specific alleles encoding the disease associated with MHC antigen are identified. In the identification of the alleles, it is assumed that the susceptibility allele is

dominant. Identification of the allele is accomplished by determining the strong positive association of a specific subtype with the disease. This may be accomplished in a number of ways, all of which are known to those skilled in the art (for example, subtyping may be accomplished by mixed lymphocyte response (MLR) typing and by primed lymphocyte testing (PLT)). Both methods are described in Weir & Blackwell, eds., *Handbook of Experimental Immunol*. It may also be accomplished by analyzing DNA restriction fragment length polymorphism (RFLP) using DNA probes that are specific for the MHC locus being examined. Methods for preparing probes for the MHC loci are known to those skilled in the art (*see, e.g.*, Gregersen *et al.*, *Proc. Natl. Acad. Sci. USA* 79:5966 (1986)).

The most complete identification of subtypes conferring disease susceptibility is accomplished by sequencing of genomic DNA of the locus, or cDNA to mRNA encoded within the locus. The DNA which is sequenced includes the section encoding the hypervariable regions of the MHC encoded polypeptide. Techniques for identifying specifically desired DNA with a probe for amplification of the desired region are known in the art, and include, for example, the polymerase chain reaction (PCR) technique. As an example, over 90% of rheumatoid arthritis patients have a haplotype of DR4(Dw4), DR4(Dw14) or DR1 (*see* Figure 1).

V. ANTIBODIES TO MHC CLASS II HYPERVARIABLE REGION

The polypeptides of the invention can also be used to raise antibodies against MHC class II molecules, e.g., haplotype specific hypervariable regions. The antibodies are useful, e.g., for treatment and diagnosis of autoimmune disease associated with a particular MHC class II allele.

Methods of producing polyclonal and monoclonal antibodies that react specifically with a MHC class II molecules are known to those of skill in the art (*see, e.g.*, Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *supra*; Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986); and Kohler & Milstein, *Nature* 256:495-497 (1975). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice (*see, e.g.*, Huse *et al.*, *Science* 246:1275-1281 (1989); Ward *et al.*, *Nature* 341:544-546 (1989)).

A number of immunogens comprising portions of MHC class II molecules, as described herein, may be used to produce antibodies specifically reactive with MHC class II molecules. For example, recombinant MHC class II molecules or an antigenic fragment

thereof can be isolated as described herein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described above, and purified as generally described above. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used an immunogen. Naturally occurring protein may also be used either in pure or impure form. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated, for subsequent use in immunoassays to measure the protein.

Methods of production of polyclonal antibodies are known to those of skill in the art. An inbred strain of mice (*e.g.*, BALB/C mice) or rabbits is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the beta subunits. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (*see,* Harlow & Lane, *supra*).

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (*see,* Kohler & Milstein, *Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by Huse, *et al.*, *Science* 246:1275-1281 (1989).

Monoclonal antibodies and polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non- MHC class II molecules, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at

least about 1 μM, preferably at least about 0.1 μM or better, and most preferably, 0.01 μM or better. Antibodies specific only for a particular molecule encoded by an allele can also be made, by subtracting out other cross-reacting molecules.

Once the specific antibodies against a MHC class II molecule are available, the MHC class II molecule can be detected by a variety of immunoassay methods. For a review of immunological and immunoassay procedures, see *Basic and Clinical Immunology* (Stites & Terr eds., 7th ed. 1991). Moreover, the immunoassays of the present invention can be performed in any of several configurations, which are reviewed extensively in Enzyme Immunoassay (Maggio, ed., 1980); and Harlow & Lane, *supra*. The antibodies raised against the MHC class II molecules of the invention, e.g., polypeptides corresponding to a hypervariable region of a molecule encoded by an allele, such as DQ or DR, can also be used therapeutically to treat autoimmune disease. Antibodies of the invention are administered according to methods known to those of skill in the art.

VI. MODEL SYSTEMS FOR TESTING ANTIGEN-ADJUVANT COMPOSITIONS OF THE INVENTION

The following are model systems for autoimmune diseases which can be used to evaluate the effects of the compositions of the invention on these conditions.

A. Systemic Lupus Erythematosus (SLE)

F_1 hybrids of autoimmune New Zealand black (NZB) mice and the phenotypically normal New Zealand White (NZW) mouse strain develop severe systemic autoimmune disease, more fulminant than that found in the parental NZB strain. These mice manifest several immune abnormalities, including antibodies to nuclear antigens and subsequent development of a fatal, immune complex-mediated glomerulonephritis with female predominance, remarkably similar to SLE in humans (Knight *et al.*, *J. Exp. Med.* 147:1653 (1978)).

In both the human and murine forms of the disease, a strong association with MHC gene products has been reported. HLA-DR2 and HLA-DR3 individuals are at a higher risk than the general population to develop SLE (Reinertsen *et al.*, *N. Engl. J. Med.* 299:515 (1970)), while in NZB/W F_1 mice (H-$2^{d/u}$), a gene linked to the h-2^u haplotype derived from the NZW parent contributes to the development of the lupus-like nephritis.

The effect of the immunogenic peptides of the invention can be measured by survival rates and by the progress of development of the symptoms, such as proteinuria and appearance of anti-DNA antibodies.

B. Myasthenia Gravis (MG)

Myasthenia gravis is one of several human autoimmune diseases linked to HLA-D (*see, e.g.*, McDevitt *et al.*, *Arth. Rheum.* 20:59 (1977)). In MG, antibodies to the acetyl choline receptors (AcChoR) impair neuromuscular transmission by mediating loss of AcChoR in the postsynaptic membrane.

SJL/J female mice are a model system for human MG. In these animals, experimental autoimmune myasthenia gravis (EAMG) is induced by immunizing the mice with soluble AcChoR protein from another species. Susceptibility to EAMG is linked in part to the MHC and has been mapped to the region within H-2 (Christadoss *et al.*, *J. Immunol.* 123:2540 (1979)).

Typically, AcChoR protein is purified from *Torpedo californica* and assayed according to the method of Waldor *et al.* (*Proc. Natl. Acad. Sci.* 80:2713 (1983)). Emulsified AcChoR, 15 μg in complete Freund adjuvant, is injected intradermally among six sites on the back, the hind foot pads, and the base of the tail. Animals are re-immunized with this same regimen 4 weeks later.

Evaluation can be made by measurement of anti-AcChoR antibodies. Anti-AcChoR antibody levels are measured by a microliter ELISA assay as described in Waldor, *et al.* (*supra*). The standard reagent volume is 50 μl per well. Reagents are usually incubated in the wells for 2 hours at room temperature. Five μg of AcChoR diluted in bicarbonate buffer, at pH 9.6, are added to each well. After incubation with AcChoR, the plates are rinsed four times with a wash solution consisting of phosphate-buffer saline containing 0.05% Tween and 0.05% NaN_3. Mouse sera are diluted in 0.01M PBS (pH 7.2), 1.5 mM $MgC1_2$, 2.0 mM β-mercaptoethanol, 0.05% Tween-80, 0.05% NaN_3 (P-Tween buffer) and incubated on the plate. After the plate is washed, β-galactosidase-conjugated sheep anti-mouse antibody diluted in P-Tween buffer is added to each well. After a final washing, the enzyme substrate, p-nitrophenyl-galctopyranoside, is added to the plate, and the degree of substrate catalysis is determined from the absorbance at 405 nm after 1 hour.

Anti-AcChoR antibodies are expected to be present in the mice immunized with AcChoR as compared to non-immunized mice. Treatment with immunogenic peptides is expected to significantly reduce the titer of anti-AcChoR antibodies in the immunized mice.

The effect of treatment with the immunogenic peptides on clinical EAMG can also be assessed. Myasthenia symptoms include a characteristic hunched posture with

drooping of the head and neck, exaggerated arching of the back, splayed limbs, abnormal walking, and difficulty in righting. Mild symptoms are present after a standard stress test, and should be ameliorated by administration of immunogenic peptides after a period of time after which antibody titer has fallen.

C. Rheumatoid Arthritis (RA)

In humans, susceptibility to rheumatoid arthritis is associated with HLA D/DR. The immune response in mice to native type II collagen has been used to establish an experimental model for arthritis with a number of histological and pathological features resembling human RA. Susceptibility to collagen-induced arthritis (CIA) in mice has been mapped to the H-2 I region, particularly the I-A subregion (*see, e.g.*, Huse *et al.*, *Fed. Proc.* 43:1820 (1984)).

Mice from a susceptible strain, DBA-1, are caused to have CIA by treatment of the mice with native type II collagen, using the technique described in Wooley & Luthra, *J. Immunol.* 134:2366 (1985).

In another model, adjuvant arthritis in rats is an experimental model for human arthritis, and a prototype of autoimmune arthritis triggered by bacterial antigens (Holoschitz *et al.*, *Prospects of Immunology* (1986); Pearson, *Arthritis Rheum.* 7:80 (1964)). The disease is the result of a cell-mediated immune response, as evidenced by its transmissibility by a clone of T cells reactive against the adjuvant (MT); the target self-antigen in the disease, based upon studies with the same cloned cells, appears to be part(s) of a proteoglycan molecule of cartilage.

Adjuvant disease in rats is produced as described by Pearson, *supra*, *i.e.*, by a single injection of Freund's adjuvant (killed tubercle bacilli or chemical fractions thereof, mineral oil, and an emulsifying agent) given into several depot sites, preferably intracutaneously, or into a paw or the base of the tail. The adjuvant is given in the absence of other antigens.

The effect of immunogenic peptide treatment on manifestations of the disease are monitored. These manifestations are histopathological, and include an acute and subacute synovitis with proliferation of synovial lining cells, predominantly a mononuclear infiltration of the articular and particular tissues, the invasion of bone and articular cartilage by connective tissue pannus, and periosteal new bone formation, especially adjacent to affected joints. In severe or chronic cases, destructive changes occur, as do fibrous or bony ankylosis.

These histopathological symptoms are expected to appear in control animals at about 12 days after sensitization to the Freund's adjuvant.

D. Insulin Dependent Diabetes Mellitus (IDDM)

IDDM is observed as a consequence of the selective destruction of insulin-secreting cells within the Islets of Langerhans of the pancreas. Involvement of the immune system in this disease is suggested by morphological evidence of early infiltration of the Islets by mononuclear cells, by the detection of anti-islet cell antibodies, by the high frequency of HLA-DR3 and -DR4 alleles in IDDM populations, and by clinical associations between IDDM and various autoimmune diseases. An animal model for spontaneous IDDM and thyroiditis has been developed in the BB rat. As in humans, the rat disease is controlled in part by the genes encoding the MHC antigens, is characterized by islet infiltration, and is associated with the presence of anti-islet antibodies. The I-E equivalent Class II MHC antigens appear to be involved in the manifestation of the autoimmune disease in the BB rat (Biotard *et al.*, *Proc. Natl. Acad. Sci. USA* 82:6627 (1985)).

Morphologically, insulitis is characterized by the presence of mononuclear inflammatory cells within the islets. Thyroiditis is characterized by focal interstitial lymphocytic infiltrate within the thyroid gland, as a minimum criterion. Most severe cases show diffuse extensive lymphocytic infiltrates, disruption of acini, fibrosis, and focal Hurthle call change (*see* Biotard *et al.*, *supra*).

Treatment of the BB rats with the immunogenic peptides of the invention is expected to ameliorate or prevent the manifestation of the clinical and morphological symptoms associated with IDDM and thyroiditis.

The NOD mouse strain (H-2K^dD^b) is another spontaneous model for autoimmune IDDM. The disease in these animals is characterized by anti-islet cell antibodies, severe insulitis, and evidence for autoimmune destruction of the β-cells (Kanazawa *et al.*, *Diabetologia* 27:113 (1984)). The disease can be passively transferred with lymphocytes and prevented by treatment with cyclosporin-A (Ikehara *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7743 (1985); Mori *et al.*, *Diabetologia* 29:244 (1986)). Untreated animals develop profound glucose intolerance and ketosis and succumb within weeks of the onset of the disease. Seventy to ninety percent of female and 20-30% of male animals develop diabetes within the first six months of life. Breeding studies have defined at least two genetic loci responsible for disease susceptibility, one of which maps to the MHC. Characterization of NOD Class II antigens at both the serological and molecular level suggest that the

susceptibility to autoimmune disease is linked to I-Aβ (Acha-Orbea & McDevitt, *Proc. Natl. Acad. Sci. USA* 84:235 (1970)). Treatment of Female NOD mice with immunogenic peptides is expected to lengthen the time before the onset of diabetes and/or to ameliorate or prevent the disease.

E. Experimental Allergic Encephalomyelitis (EAE)

Experimental allergic encephalomyelitis (EAE) is an induced autoimmune disease of the central nervous system which mimics in many respects the human multiple sclerosis (MS) disease. The disease can be induced in many species, including mice and rats.

The disease is characterized by the acute onset of paralysis. Perivascular infiltration by mononuclear cells in the CNS is observed in both mice and rats. Methods of inducing the disease, as well as symptomology, are reviewed by Aranson, in *The Autoimmune Diseases* pp. 399-427, eds. Rose & Mackay (1985), and in Acha-Orbea *et al.*, *Ann. Rev. Imm.* 7:377-405 (1989)).

One of the genes mediating susceptibility is localized in the MHC class II region (Moore *et al.*, *J. Immunol.* 124:1815-1820 (1980)). The best analyzed encephalitogenic protein is myelin basic protein (MBP), but other encephalitogenic antigens are found in the brain. The immunogenic epitopes have been mapped (*see* Acha-Orbea *et al.*, *supra.*). In the PL mouse strains (H-2u) two encephalitogenic peptides in MBP have been characterized: MBP peptide p35-47 (MBP 35-47), and acetylated (MBP 1-9). In humans, preferred autoantigenic peptides for the treatment of MS comprise amino acids 84-102 and 148-162 of MBP.

The effect of the immunogenic peptides of the invention on ameliorating and preventing disease symptoms in individuals in which EAE has been induced can be measured by survival rates, and by the progress of the development of symptoms. An example of the use of immunogenic compositions in the treatment of EAE is provided below.

VII. PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

The polypeptides, adjuvant-polypeptide compositions, and antibodies of the present invention and pharmaceutical compositions thereof are useful for administration to mammals, particularly humans, to treat and/or prevent deleterious immune responses. Suitable formulations are found in *Remington, Pharmaceutical Sciences*, 17th ed. (1985).

The immunogenic polypeptides and polypeptide-adjuvant compositions of the invention are administered prophylactically or to an individual already suffering from the

disease. The compositions are administered to a patient in an amount sufficient to elicit an effective immune response to the MHC molecule from which the peptides are derived. An amount adequate to accomplish this is defined as "therapeutically effective dose" or "immunogenically effective dose." Amounts effective for this use will depend on, *e.g.*, the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization dose (that is for therapeutic or prophylactic administration) from about 0.01 mg to about 50 mg per 70 kilogram patient, more commonly from about 0.5-1 mg to about 10-15 mg per 70 kg of body weight. Boosting dosages are typically from about 0.01 mg to about 50 mg of peptide, more commonly about 0.5-1 mg to about 10-15 mg, using a boosting regimen over weeks to months depending upon the patient's response and condition. A suitable protocol would include injection at time 0, 2, 6, 8, 10 and 14 weeks, followed by booster injections at 24 and 28 weeks. Booster injections can be from one, two, three, four, five or more. Initial and booster injection amounts and timing are determined based on the judgment of the physician and the antigen being administered. In one embodiment, the initial and booster dose is 1.3 mg, 4 mg, or 13 mg, administered via intramuscular injection, with at least one and up to 3 booster injections at 8 week intervals, or at least one and up to 4 booster injections at 6 week intervals.

It must be kept in mind that the peptides, antibodies, and compositions of the present invention may generally be employed in serious disease states, that is, life-threatening or potentially life-threatening situations. In such cases, in view of the minimization of extraneous substances and the relative nontoxic nature of the peptides, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions.

For therapeutic use, administration should begin at the first sign of autoimmune or allergic disease. This is followed by boosting doses at least until symptoms are substantially abated and for a period thereafter. In some circumstances, loading doses followed by boosting doses may be required. The resulting immune response helps to cure or at least partially arrest symptoms and/or complications. Vaccine compositions containing the peptides, antibodies, or DNA encoding the polypeptides are administered prophylactically to a patient susceptible to or otherwise at risk of developing the disease to elicit an immune response against the target MHC antigen. Such compositions can also be administered to treat patients who have already developed disease, and to treat further disease development.

The pharmaceutical compositions are intended for parenteral or oral administration. Preferably, the pharmaceutical compositions are administered parenterally, *e.g.*, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier or an oil-in-water emulsion, together with the CWS adjuvant and additional additives, as needed. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides of the invention, more preferably at a concentration of 25%-75%.

As noted above, the compositions are intended to induce an immune response to the peptides. Thus, compositions and methods of administration suitable for maximizing the immune response are preferred. For instance, peptides may be introduced into a host, including a human, linked to a carrier or as a homopolymer or heteropolymer of active peptide units. Alternatively, a "cocktail" of polypeptides can be used. A mixture of more than one polypeptide has the advantage of increased immunological reaction and, where different peptides are used to make up the polymer, the additional ability to induce antibodies to a number of epitopes. For instance, polypeptides comprising sequences from hypervariable regions of the α and β chains may be used in combination. Useful carriers are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(lysine:glutamic acid), influenza, hepatitis B virus core protein, hepatitis B virus recombinant vaccine and the like.

The use of more than one polypeptide or antibody is particularly useful to enhance the immune response against polypeptides of the invention. As demonstrated below, although the polypeptides may be derived from self MHC class II molecules expressed in the patient, they can induce an immune response. In some instances, the immune response to the self polypeptide may, however, not be sufficiently strong. In these instances, it may be necessary to break the tolerance to the polypeptide. The compositions may comprise one or more of the foreign polypeptides that are sufficiently similar to the self polypeptides to induce an immune response against both the foreign and self polypeptides (*see*, Mamula *et al.*, *J. Immunol.* 149:789-795 (1992)). Suitable proteins include synthetic polypeptides designed for this purpose or polypeptide sequences from homologous proteins from natural sources, such as proteins encoded by a different allele at the same locus as the self polypeptide.

An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres or nanocapsules; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

The compositions may also include a *Mycobacterium* species CWS adjuvant, as described above. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide.

Certain adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL® adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG

dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science 273*:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins . Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β-escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamelar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol[R] to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other formulations comprise an oil-in-water emulsion and tocopherol. Another adjuvant formulation employs QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn®) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula (I): $HO(CH_2CH_2O)_n$-A-R,
wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein n is between 1 and 50, preferably 4-24, most preferably 9; the R component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and A is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

In another illustrative embodiment, biodegradable microspheres (*e.g.*, polylactate polyglycolate) are employed as carriers for the compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344, 5,407,609 and 5,942,252. Modified hepatitis B core protein carrier systems. such as described in WO/99 40934, and references cited therein, will also be useful for many applications. Another illustrative carrier/delivery system employs a carrier comprising particulate-protein

complexes, such as those described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

The concentration of immunogenic peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually from at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

The peptides can also be used for diagnostic purposes. For instance, they can be used to screen for autoantibodies to ensure that the vaccination has been effective. In another embodiment, the peptides and compositions of the invention can be used to determine whether a patient is suffering from an autoimmune disease, or that the autoimmune disease is progressing. The compositions of the invention, either with or without adjuvant, can be used, *e.g.*, to elicit a DTH reaction, *e.g.*, in a skin test, as a diagnostic or prognostic marker of disease. In one embodiment, the peptides of the invention are administered to a mouse footpad, along with APC and T cells from a patient to monitor for human DTH responsiveness specific for the peptide.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

VIII. EXAMPLES

Example I: Administration of IA^s β chain 57-76 peptide with *M. phlei* CWS-SE adjuvant to EAE mice

The method described below has been previously used to test the effect of MHC class II polypeptides of the invention with the adjuvant CFA in a mouse EAE model. Due to its high immunogenicity, CFA is, however, not used in humans. Alum adjuvant was previously used in humans with the MHC polypeptides of the invention.

SJL mice (8-12 weeks old) were immunized subcutaneously on the dorsum with 200 μg IA^s β chain 57-76 peptide (*see* U.S. Patent No. 6,045,796) in the presence of complete Freund's adjuvant (CFA) or *M. phlei* CWS-SE adjuvant. A 3 ml stock solution of *M. phlei* CWS adjuvant (made by Corixa Corporation, Hamilton, Montana, catalogue number 250 and 251) contains 1500 μg/ml of CWS, 81.15 mg/ml of squalane, 20 mg/ml of surfactant such as Tween-80, and 1 ml of water for injection. The peptide-adjuvant composition contains a 1:10 dilution of CWS stock, and a 1:10 dilution of stock peptide (10 mg/ml) in PBS. 200 μl are injected per mouse (2 injections of 100 μl each). Thus, *e.g.*, for ten mice, a total of 2 ml peptide-adjuvant composition is prepared, containing 0.2 ml of stock peptide, 0.2 ml of stock CWS, and 1.6 ml of PBS.

Seven days later, animals were re-vaccinated with equivalent amounts of the same peptide. Control animals were vaccinated with CFA alone or with CWS alone under the same conditions.

Two weeks after the last administration of the peptide, disease was induced by subcutaneous injection of PLP 139-152 peptide in CFA. Briefly the peptide (200 μg/mouse) was dissolved in PBS and emulsified with an equal volume of CFA. Each mouse received 0.15 ml of the emulsion, which was administered subcutaneously and distributed over three sites on the flank and base of the tail.

Approximately 9-12 days after disease induction, animals were observed daily for the onset of neurological dysfunction. Disease was graded by trained technicians as follows: 0, normal; 0.5, stiff tail; 1, limp tail; 1.5, limp tail with inability to rightness; 2, paralysis of one limb; 2.5 paralysis of one limb and weakness of one other limb; 3, complete paralysis of both hind limbs; 4, moribund; 5, death. Mice are usually followed for up to 60 days for relapses. As shown in Figure 5 and 6, administration of the IA^s β chain 57-76 peptide in the presence of CWS significantly reduced the incidence of EAE. Adjuvant alone did not have a protective effect, indicating the specificity of the immune response.

Example II: Administration of IA^s β chain 57-76 peptide with *M. phlei* CWS-AQ adjuvant to EAE mice

SJL mice (8-12 weeks old) were immunized subcutaneously on the dorsum with 200 μg IA^s β chain 57-76 peptide (*see* U.S. Patent No. 6,045,796) in the presence of complete Freund's adjuvant (CFA) *M. phlei* CWS-SE adjuvant or *M. phlei* CWS-AQ adjuvant. CWS-SE was made as described above. A 3 ml stock solution of *M. phlei* CWS-

AQ adjuvant (made by Corixa Corporation, Hamilton, Montana) contains 1000 μg/ml of CWS, 0.03% Tween-80 (V/V) and 0.9% saline (W.V) in water for injection. The peptide-adjuvant composition contains a 1:10 dilution of CWS stock (SE or AQ), and a 1:10 dilution of stock peptide (10 mg/ml) in PBS. 200 μl are injected per mouse (2 injections of 100 μl each). Thus, *e.g.*, for ten mice, a total of 2 ml peptide-adjuvant composition is prepared, containing 0.2 ml of stock peptide, 0.2 ml of stock CWS, and 1.6 ml of PBS.

Seven days later, animals were re-vaccinated with equivalent amounts of the same peptide. Control animals were vaccinated with CFA alone or with CWS alone under the same conditions.

Two weeks after the last administration of the peptide, disease was induced by subcutaneous injection of PLP 139-152 peptide in CFA. Briefly the peptide (200 μg/mouse) was dissolved in PBS and emulsified with an equal volume of CFA. Each mouse received 0.15 ml of the emulsion, which was administered subcutaneously and distributed over three sites on the flank and base of the tail.

Approximately 9-12 days after disease induction, animals were observed daily for the onset of neurological dysfunction. Disease was graded by trained technicians as follows: 0, normal; 0.5, stiff tail; 1, limp tail; 1.5, limp tail with inability to rightness; 2, paralysis of one limb; 2.5 paralysis of one limb and weakness of one other limb; 3, complete paralysis of both hind limbs; 4, moribund; 5, death. Mice are usually followed for up to 60 days for relapses. As shown in Figures 7-10, administration of the IA^s β chain 57-76 peptide in the presence of CW-AQS significantly reduced the incidence of EAE. Adjuvant alone did not have a protective effect, indicating the specificity of the immune response.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising an isolated immunogenic MHC class II polypeptide, wherein the polypeptide has an amino acid sequence from a hypervariable region of an MHC class II molecule, in combination with an adjuvant comprising isolated cell wall skeleton from a *Mycobacterium* species.

2. The composition of claim 1, wherein the *Mycobacterium* is *Mycobacterium phlei.*

3. The composition of claim 1, wherein the composition further comprises an oil-in-water emulsion.

4. The composition of claim 3, wherein the oil is squalane oil.

5. The composition of claim 1, wherein the adjuvant further comprises a detergent.

6. The composition of claim 5, wherein the detergent is Tween-80.

7. The composition of claim 1, wherein the composition further comprises a squalene oil-in-water emulsion and Tween-80.

8. The composition of claim 1, wherein the MHC class II molecule is from a human.

9. The composition of claim 1, wherein the MHC class II molecule is from a mouse.

10. The composition of claim 1, wherein the polypeptide is about 15 to about 25 amino acids in length.

11. The composition of claim 1, wherein the MHC class II molecule is an MHC class II β chain.

12. The composition of claim 11, wherein the MHC class II molecule is a human HLA-DR β chain.

13. The composition of claim 12, wherein the MHC class II molecule is a human HLA-DR4 β chain.

14. The composition of claim 12, wherein the MHC class II molecule is encoded by the HLA-DR B1*0401 allele or the HLA-DR B1*0101 allele.

15. The composition of claim 12, wherein the polypeptide comprises an amino acid sequence corresponding to amino acids 57-76 of the HLA-DR β chain.

16. The composition of claim 15, wherein the polypeptide further comprises a cysteine amino acid residue at the N-terminus.

17. The composition of claim 15, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of DAEYWNSQKDLLEQKRAAVD or DAEYWNSQKDLLEQRRAAVD.

18. The composition of claim 11, wherein the MHC class II molecule is a human HLA-DQ β chain.

19. The composition of claim 18, wherein the MHC class II molecule is encoded by the HLA-DQ B1*0401 allele, the HLA-DQ B1*0303(2) allele, the HLA-DQ B1*0201 allele or the HLA-DQ B1*0302 allele.

20. The composition of claim 18, wherein the polypeptide comprises an amino acid sequence corresponding to amino acids 43-62 of the human HLA-DQ β chain.

21. The composition of claim 20, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, and DVGVYRAVTPLGPPAAEYWN.

22. The composition of claim 18, wherein the polypeptide comprises an amino acid sequence corresponding to amino acids 54-76 of the human HLA-DQ β chain.

23. The composition of claim 22, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of

GRLDAEYWNSQKDILEEDRASVD, GPPDAEYWNSQKEVLERTRAELD, GLPAAEYWNSQKDILERKRAAVD, and GPPAAEYWNSQKEVLERTRAELD.

24. The composition of claim 1, wherein the polypeptide comprises the amino acid sequence DAEYWNSQKDLLEQKRAAVD, DAEYWNSQKDLLEQRRAAVD, DVGEYRAVTELGRHSAEYYN, DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, DVGVYRAVTPLGPPAAEYWN, GRHSAEYYNKQYLERTRAELDTA, GRLDAEYWNSQKDILEEDRASVD, GPPDAEYWNSQKEVLERTRAELD, GLPAAEYWNSQKDILERKRAAVD, or GPPAAEYWNSQKEVLERTRAELD.

25. The composition of claim 1, wherein the polypeptide has an acetylated N-terminal amino acid.

26. The composition of claim 1, wherein the polypeptide has an amino acid sequence from an MHC class II molecule encoded by an allele associated with an autoimmune disease.

27. The composition of claim 26, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

28. A pharmaceutical composition comprising an isolated immunogenic MHC class II polypeptide, wherein the polypeptide has the amino acid sequence DAEYWNSQKDLLEQKRAAVD, DAEYWNSQKDLLEQRRAAVD, DVGEYRAVTELGRHSAEYYN, DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, DVGVYRAVTPLGPPAAEYWN, GRHSAEYYNKQYLERTRAELDTA, GRLDAEYWNSQKDILEEDRASVD, GPPDAEYWNSQKEVLERTRAELD, GLPAAEYWNSQKDILERKRAAVD, or GPPAAEYWNSQKEVLERTRAELD in combination with an adjuvant comprising squalane oil and an immunostimulatory compound, wherein the immunostimulatory compound is isolated cell wall skeleton from *Mycobacterium phlei*.

29. A method of eliciting an immune response in a subject, the method comprising the step of administering to the subject an immunologically effective amount of a

pharmaceutical composition comprising an isolated immunogenic MHC class II polypeptide, wherein the polypeptide has an amino acid sequence from a hypervariable region of an MHC class II molecule, in combination with an adjuvant comprising isolated cell wall skeleton from a *Mycobacterium* species.

30. The method of claim 29, wherein the subject is a human.

31. The method of claim 29, wherein the composition is administered intramuscularly.

32. The method of claim 29, wherein the composition is administered prophylactically.

33. The method of claim 29, wherein the composition is administered to a subject having an autoimmune disease.

34. The method of claim 33, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

35. The method of claim 29, wherein the composition is administered in an immunologically effective amount of about 1 mg polypeptide to about 15 mg polypeptide.

36. The method of claim 29, wherein the composition is administered in an immunologically effective amount of about 0.1 mg polypeptide to about 10 mg polypeptide.

37. The method of claim 29, wherein the *Mycobacterium* species is *Mycobacterium phlei*.

38. The method of claim 29, wherein the composition further comprises an oil-in-water emulsion.

39. The method of claim 38, wherein the oil is squalane oil.

40. The method of claim 29, wherein the polypeptide is about 15 to about 25 amino acids in length.

41. The method of claim 29, wherein the MHC class II molecule is an MHC class II β chain.

42. The method of claim 41, wherein the MHC class II molecule is a human HLA-DR β chain.

43. The method of claim 42, wherein the MHC class II molecule is a human HLA-DR4 β chain.

44. The method of claim 42, wherein the MHC class II molecule is encoded by the HLA-DR B1*0401 allele or the HLA-DR B1*0101 allele.

45. The method of claim 42, wherein the polypeptide comprises an amino acid sequence corresponding to amino acids 57-76 of the HLA-DR β chain.

46. The method of claim 41, wherein the MHC class II molecule is the human HLA-DQ β chain.

47. The method of claim 46, wherein the MHC class II molecule is encoded by the HLA-DQ B1*0401 allele, the HLA-DQ B1*0303(2) allele, the HLA-DQ B1*0201 allele or the HLA-DQ B1*0302 allele.

48. The method of claim 46, wherein the polypeptide comprises an amino acid sequence corresponding to amino acids 43-62 of the human HLA-DQ β chain.

49. The method of claim 46, wherein the polypeptide comprises an amino acid sequence corresponding to amino acids 54-76 of the human HLA-DQ β chain.

50. The method of claim 29, wherein the polypeptide comprises the amino acid sequence DAEYWNSQKDLLEQKRAAVD, DAEYWNSQKDLLEQRRAAVD, DVGEYRAVTELGRHSAEYYN, DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, DVGVYRAVTPLGPPAAEYWN, GRHSAEYYNKQYLERTRAELDTA, GRLDAEYWNSQKDILEEDRASVD, GPPDAEYWNSQKEVLERTRAELD, GLPAAEYWNSQKDILERKRAAVD, or GPPAAEYWNSQKEVLERTRAELD.

51. The method of claim 29, wherein the polypeptide has an amino acid sequence from an MHC class II molecule encoded by an allele associated with an autoimmune disease.

52. The method of claim 51, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

53. A pharmaceutical composition comprising MHC class II polypeptide comprising a sequence corresponding to amino acids 43-62 of the human HLA-DQ β chain, and a pharmaceutically acceptable carrier.

54. A pharmaceutical composition according to claim 53, wherein the MHC class II polypeptide comprises a sequence selected from the group consisting of DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, and DVGVYRAVTPLGPPAAEYWN.

55. A pharmaceutical composition according to claim 53, further comprising an adjuvant.

56. A method of eliciting an immune response in a subject, the method comprising the step of administering to the subject an immunologically effective amount of the pharmaceutical composition of claim 53.

57. The method of claim 56, wherein the composition is administered intramuscularly.

58. The method of claim 56, wherein the composition is administered prophylactically.

59. The method of claim 56, wherein the subject is a human.

60. The method of claim 56, wherein the composition is administered to a subject having an autoimmune disease.

61. The method of claim 60, wherein the autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, insulin dependent diabetes mellitus, systemic lupus erythematosus, and multiple sclerosis.

62. An isolated MHC class II polypeptide comprising a sequence corresponding to amino acids 43-62 of the human HLA-DQ β chain.

63. The compound of claim 62, wherein the MHC class II polypeptide comprises a sequence selected from the group consisting of DVGVYRAVTPLGRLDAEYWN, DVGVYRAVTPLGPPDAEYWN, DVGEFRAVTLLGLPAAEYWN, and DVGVYRAVTPLGPPAAEYWN.

CAUCASIAN DQ/DR HAPLOTYPES[1]

#	HPTYP FREQ.%[2]	DQ	DQB1	DQA1[3]	DRB1	DRB3	DRB4	D	DISEASE ASSOCIATION[4]
1.	20	w5(w1)	1.1	1a	1	ne	ne	w1	IDDM (Major), RA (Minor)
2.		w5(w1)	1.1	1a	1	ne	ne	w20	
3.	26	w6(w1)	1.2	1b	w15(2)	ne	ne	w2	CPMS, MG (T+)
4.	1.5	w6(w1)	1.12	1c	w15(2)	ne	ne	w12	IDDM(-)
5.	1.5	w5(w1)	1.1	?	w16(2)	ne	ne	w21(AZH)	IDDM(+), MG(T-)
6.	?	w7(w3)	3.1	?	w16(2)	ne	ne	w22	
7.	22	w2	?	?	w17(3)	24(52)	ne	w3	
8.		w2	?	?	w17(3)	25(52)	ne	w3	IDDM(+), MG(T-)
9.	?	w4(Wa)	Wa	?	w18(3)	? (52)	ne	?	
10.	9	w8(w3)	3.2	3	4	ne	53	w4(4.2)	IDDM*(+)(Major), RA† (Major), CPMS
11.	5	w7(w3)	3.1	3	4	ne	53	w4(4.1)	
12.	3	w8(w3)	3.2	3	4	ne	53	w10	IDDM*(+) (Major), CPMS
13.	7	w7(w3)	3.1	3	4	ne	53	w13	
14.	14	w8(w3)	3.2	3	4	ne	53	w14	IDDM*(+) (Major), RA† (Major), CPMS
15.	0.5	w4(Wa)	Wa	?	4	ne	53	w15	
16.	15	w7(w3)	3.1	2	w11(5)	25(52)	ne	w5	
17.		w7(w3)	3.1	2	w12(5)	25(52)	ne	B6	
18.	10	w5(w1)	1.18	1c	w(13)(w6)	24(52)	ne	w18	
19.		w5(w1)	1.18	1c	w(13)(w6)	25(52)	ne	w18	
20.	3	w5(w1)	1.19	1b	w(13)(w6)	26(52)	ne	w19	IDDM (Minor)
21.	3	w6(w1)	1.9	1a	w(14)(w6)	25(52)	ne	w9	
22.	?	w6(w1)	1.16	2	w(14)(w6)	24(52)	ne	w16	RA (Minor)
23.	1	w9(w3)	3.3	3	7	ne	53	w11	
24.	27	w2	2	3	7	ne	53	w17	
25.	6	w4(Wa)	Wa	1b	ne	w8/52	ne	w8	
26.	2	? (w3)	?	1b	ne	w8/52	ne	w8	
27.	1	w9(w3)	3.3	3	9	ne	53	w23	
28.	?	w5(w1)	1.1	1a	w10	?	?	?	

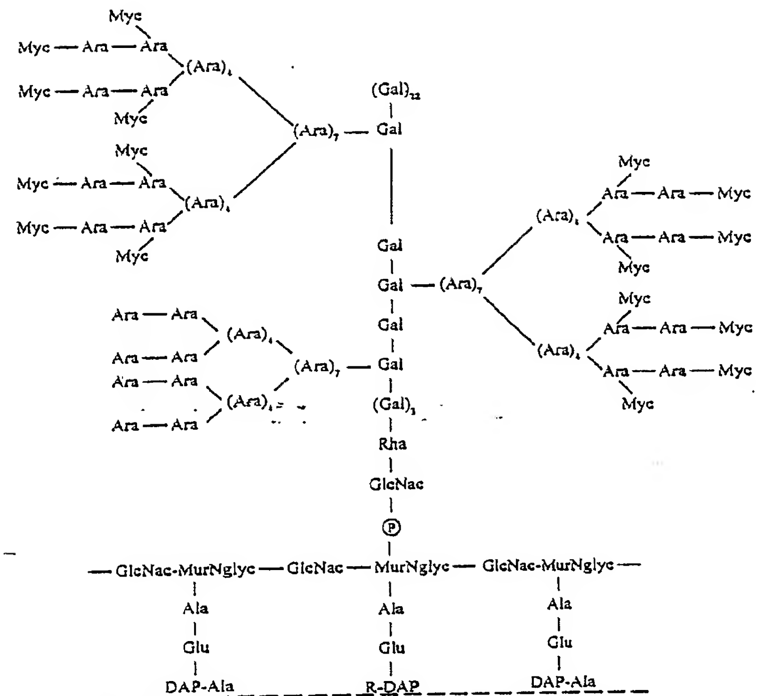
ne: Not Expressed
T-: Thymoma - Negative
T+: Thymoma-Positive
CPMS: Chronic-Progressive Multiple Sclerosis
IDDM: Insulin-Dependent Diabetes Mellitus
MG: Myasthenia Gravis
RA: Rheumatoid Arthritis

1. Nomenclature Committee on Leucocyte Antigens. Tissue Antigens, 32:177-187 (1988).
2. Todd, J.A., et al., *HLA-DQβ Gene Contributes to Susceptibility and Resistence to Insulin-Dependent Diabetes Mellitus.* Nature, 329:599-604 (1987).
3. Bidwell, J., *DNA-RFLP Analysis and Genotyping of HLA-DR & DQ Antigens.* Immunology Today, 9:18-23 (1988).
4. IDDM: See #3 above.
   RA: Winchester, R., *Genetics of Autoimmune Diseases.* Current Opinions in Immunology, 1(No. 4):701-707 (1989).
   CPMS & MG: Möller et al., *Polymorphism of HLA Class II Genes in Various Diseases.* In: Molecular Basis of the Immune Response, Annals of the New York Academy of Sciences, 546: 143-150(1988).

* About 90% of IDDM patients have haplotype #10, 12, or 14 (Dw4, Dw10 or Dw14).

† Over 90% of RA patients have haplotype #10 or 14 (Dw4 or Dw14).

Figure 1



Myc = mycolic acid
Ara = arabinose
Gal = galactose
Rha = Rhamnose
MurNglyc = N-glycoylmuramic acid
Ala = alanine
Ⓟ = phosphodiester
Glu = glutamic acid
DAP = diaminopimelic acid
GlcNAc = N-acetylglucosamine
R = another peptidoglycan unit
(cross-liked to DAP of another unit)

Figure 2

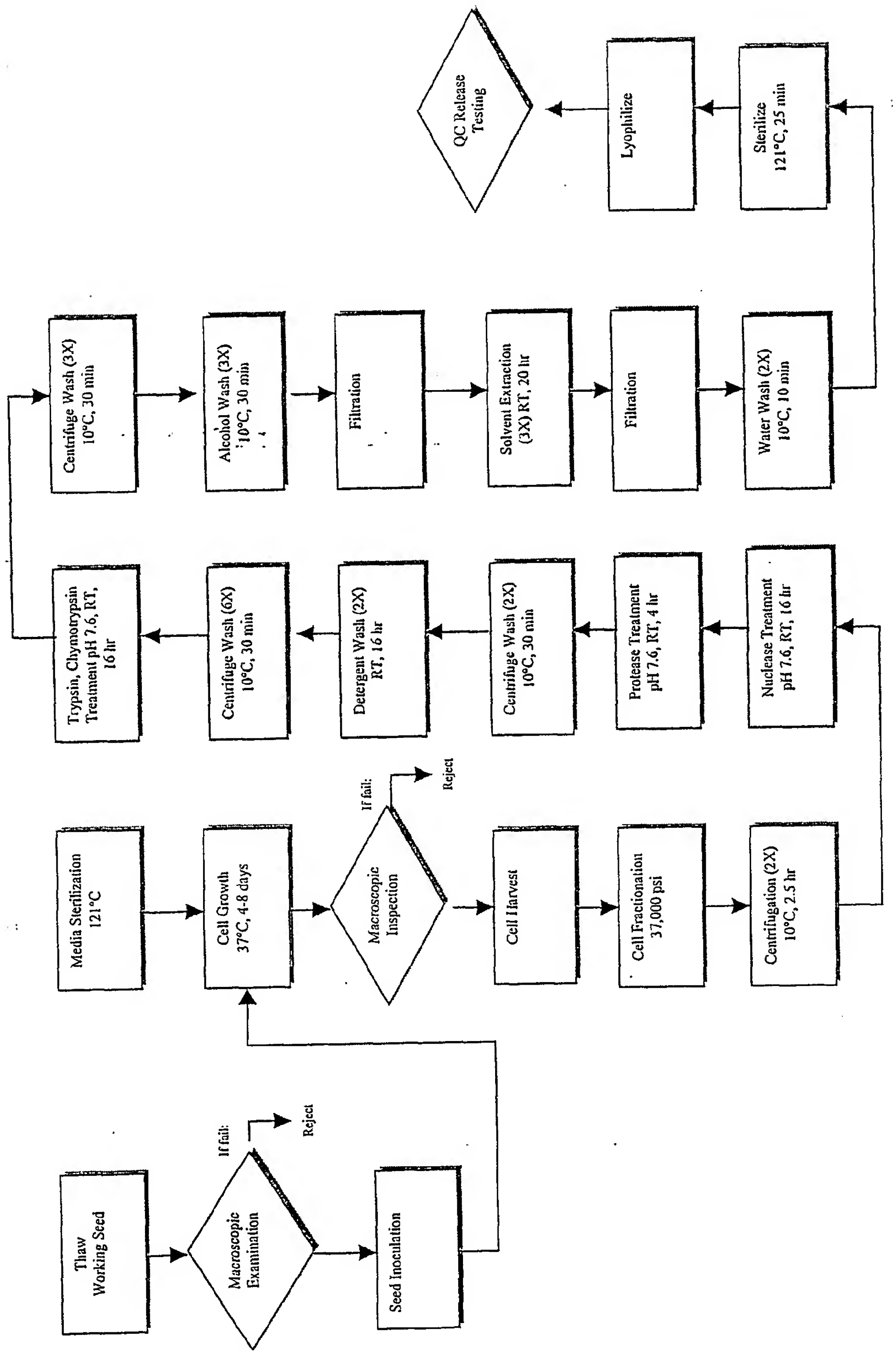

Thaw
Working Seed
Macroscopic
Examination
If fail:
Reject
Seed Inoculation
Media Sterilization
121°C
Cell Growth
37°C, 4-8 days
Macroscopic
Inspection
If fail:
Reject
Cell Harvest
Cell Fractionation
37,000 psi
Centrifugation (2X)
10°C, 2.5 hr
Nuclease Treatment
pH 7.6, RT, 16 hr
Protease Treatment
pH 7.6, RT, 4 hr
Centrifuge Wash (2X)
10°C, 30 min
Detergent Wash (2X)
RT, 16 hr
Centrifuge Wash (6X)
10°C, 30 min
Trypsin, Chymotrypsin
Treatment pH 7.6, RT,
16 hr
Centrifuge Wash (3X)
10°C, 30 min
Alcohol Wash (3X)
10°C, 30 min
Filtration
Solvent Extraction
(3X) RT, 20 hr
Filtration
Water Wash (2X)
10°C, 10 min
Sterilize
121°C, 25 min
Lyophilize
QC Release
Testing

Figure 3

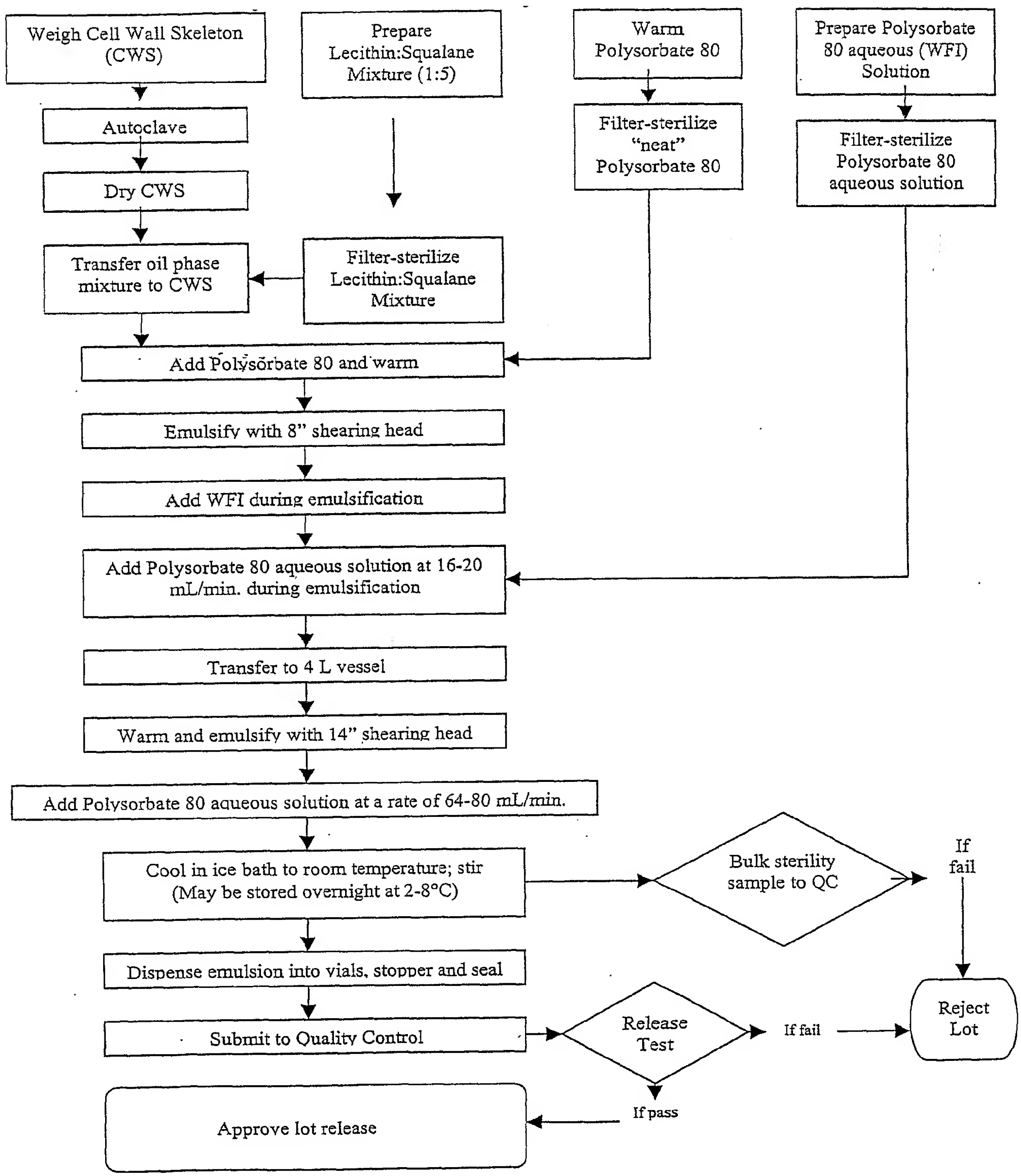
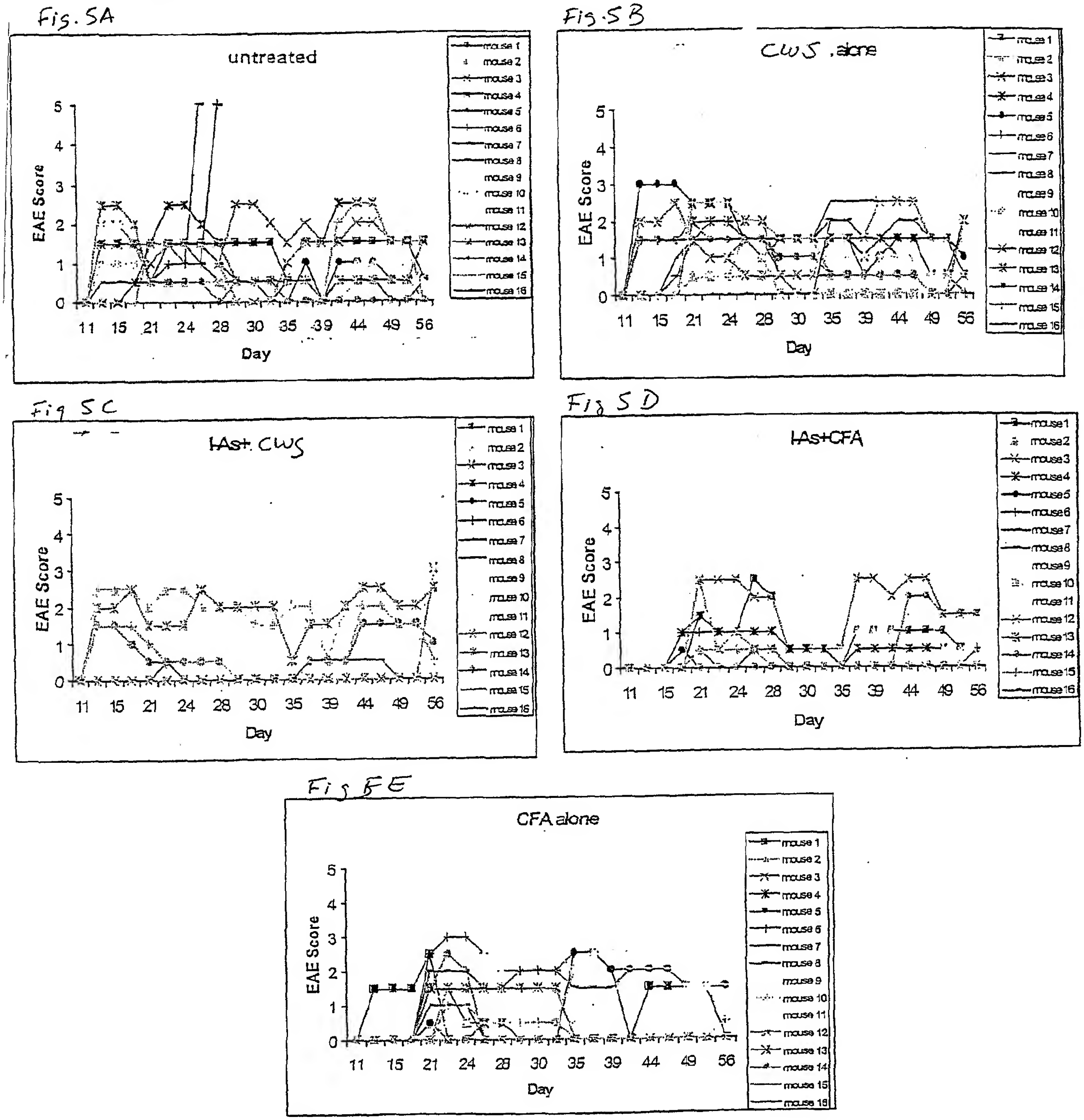

Weigh Cell Wall Skeleton (CWS)
Autoclave
Dry CWS
Transfer oil phase mixture to CWS
Prepare Lecithin:Squalane Mixture (1:5)
Filter-sterilize Lecithin:Squalane Mixture
Warm Polysorbate 80
Filter-sterilize "neat" Polysorbate 80
Prepare Polysorbate 80 aqueous (WFI) Solution
Filter-sterilize Polysorbate 80 aqueous solution
Add Polysorbate 80 and warm
Emulsify with 8" shearing head
Add WFI during emulsification
Add Polysorbate 80 aqueous solution at 16-20 mL/min. during emulsification
Transfer to 4 L vessel
Warm and emulsify with 14" shearing head
Add Polysorbate 80 aqueous solution at a rate of 64-80 mL/min.
Cool in ice bath to room temperature; stir (May be stored overnight at 2-8°C)
Bulk sterility sample to QC
If fail
Dispense emulsion into vials, stopper and seal
Submit to Quality Control
Release Test
If fail
Reject Lot
If pass
Approve lot release

Figure 4

Figure 5

ANERVAX #4 EXP: EFFECT OF DETOX W/O MPL AS ADJUVANT FOR ANERVAX PEPTIDE



Strain/Group/ # of mice	Vaccination Day −21 Day −14	EAE induction Day 0	EAE incidence day +56 # of mice with disease/total of mice
SJL/ 16 mice	I-As β chain peptide/CFA 200 ug/dose	PLP 139-151 200 ug/CFA/ mouse/150 μl	7/16 (43.7%)
SJL/ 16 mice	I-As β chain peptide/CWS 200 ug/dose	PLP 139-151 200 ug/CFA/ mouse/150 μl	5/16 (31.2%)*
SJL/ 16 mice	CFA alone	PLP 139-151 200 ug/CFA/ mouse/150 μl	13/16 (81.2%)
SJL/ 16 mice	CWS alone	PLP 139-151 200 ug/CFA/ mouse/150 μl	13/16 (81.2%)
SJL/ 16 mice	untreated	PLP 139-151 200 ug/CFA/ mouse/150 μl	16/16 (100%) *

* p value< 0.0001

Figure 6

Figure 7

DAEYWNSQKDLLEQKRAAVD (SEQ ID NO:1) (human)

DAEYWNSQKDLLEQRRAAVD (SEQ ID NO:2) (human)

DVGEYRAVTELGRHSAEYYN (SEQ ID NO:3) (mouse)

DVGVYRAVTPLGRLDAEYWN (SEQ ID NO:4) (human)

DVGVYRAVTPLGPPDAEYWN (SEQ ID NO:5) (human)

DVGEFRAVTLLGLPAAEYWN (SEQ ID NO:6) (human)

DVGVYRAVTPLGPPAAEYWN (SEQ ID NO:7) (human)

GRHSAEYYNKQYLERTRAELDTA (SEQ ID NO:8) (mouse)

GRLDAEYWNSQKDILEEDRASVD (SEQ ID NO:9) (human)

GPPDAEYWNSQKEVLERTRAELD (SEQ ID NO:10) (human)

GLPAAEYWNSQKDILERKRAAVD (SEQ ID NO:11) (human)

GPPAAEYWNSQKEVLERTRAELD (SEQ ID NO:12) (human)

Figure 8

CWS-AQ + IAs β chain peptide reduces the incidence of EAE in SJL mice. (EXP. #1)

Strain/Group/ # of mice	Vaccination Day –21 Day –14	EAE induction Day 0	# of mice with disease/total # of mice Day 58
SJL/ 20 mice Group A	Linear I-As b chain peptide/CFA 200 ug/200 μl	PLP 139-151 200 ug/CFA/ mouse/150 μl	8/20 (40%)
SJL/ 20 mice Group B	Linear I-As b peptide/ CWS-SE Lot# PD 091599-575-129 200 ug/200 μl	PLP 139-151 200 ug/CFA/ mouse/150 μl	14/20 (70%)
SJL/ 20 mice Group C	Linear I-As b peptide/ CWS-AQ Lot # PD 121-799-575-165 200 ug/200 μl	PLP 139-151 200 ug/CFA/ mouse/150 μl	6/18 (28%)*
SJL/ 20 mice Group D	CWS-SE alone 200 μl	PLP 139-151 200 ug/CFA/ mouse/150 μl	12/20 (60%)
SJL/20 mice Group E	CWS-AQ alone 200 μl	PLP 139-151 200 ug/CFA/ mouse/150 μl	12/20 (60%)
SJL/ 20 mice Group F	untreated	PLP 139-151 200 ug/CFA/ mouse/150 μl	13/19 (68%)

Figure 9

CWS-AQ + IAs β chain peptide reduces the incidence of EAE in SJL mice. (EXP. #2)

Strain/Group/ # of mice	Vaccination Day -21 Day -14	EAE induction Day 0	Incidence of disease Day +57
SJL/ 16 mice Group A	Linear I-As b chain peptide/CFA Linear I-As b chain peptide/CFA 200 ug/200 µl	PLP 139-151 200 µg/CFA/ mouse/150 µl	4/16 (25%)
SJL/ 16 mice Group B	Linear I-As b chain peptide/CWS-AQ	PLP 139-151 200 µg/CFA/ mouse/150 µl	7/16* (43%)
SJL/ 16 mice Group C	Linear I-As b chain peptide/CWS-SE	PLP 139-151 200 µg/CFA/ mouse/150 µl	12/16 (75%)
SJL/ 16 mice Group D	Linear I-As b chain peptide/DetoX	PLP 139-151 200 µg/CFA/ mouse/150 µl	15/16 (93%)
SJL/ 16 mice Group E	untreated	PLP 139-151 200 µg/CFA/ mouse/150 µl	16/16 (100%)

* $P < 0.008$

Figure 10

Effect of different Adjuvants + IAs peptide in the EAE model: summary table

Vaccination protocol* (day –21, day –14)	EAE incidence		
	Exp. #1	Exp. #2	Exp. #3
Untreated	68%	100%	92%
AnervaX + CFA	40%	25%	
AnervaX + CWS-AQ	28%	43%	17%
CWS-AQ alone	60%	-	-
AnervaX + CWS-SE	70%	75%	-
CWS-SE alone	60%	-	-
AnervaX + DetoX	-	93%	-

* (disease is induced at day 0 with PLP 139-151 in CFA)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11298

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 45/00
US CL : 424/278.1, 279.1, 282.1, 283.1; 514/12, 885
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/278.1, 279.1, 282.1, 283.1; 514/12, 885

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS, *medline caplus embase biosis*
West Patents

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	SAYEGH et al. Induction of immunity and oral tolerance with polymorphic class II major histocompatibility complex allopeptides in the rat. Proc. Natl. Acad. Sci., USA. August 1992, Vol. 89, pages 7762-7766, entire document.	1-3, 10-11, 29, 32, 36-38, 40-41 ------- 4-7 and 39
A	SPACK et al. Therapeutic and Diagnostic Applications of Solubilized MHC II Peptide Complexes in Multiple Sclerosis. Journal of Neuroimmunology. September 1998, Vol. 90 No. 1, page 99, Abstract No. 563.	1-14, 18-19, 25-27, 29-44, 46-47, 51-52
A	ZHU et al. Recombinant MHC Class II peptide Complexes Prevent Experimental Autoimmune Encephalomyelitis. FASEB Journal. April 2000, Vol. 14, No. 6 page A1247, Abstract No. 200.5.	1=14, 18-19, 25-27, 29-44, 46-47, 51-52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 MAY 2001	09 JUL 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Joyce Bridgers for AMY DECLOUX
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT	International application No. PCT/US01/11298

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAVANAUGH et al. Allele and Antigen Specific Treatment of Rheumatoid Arthritis: A Double Blind, Placebo Controlled Phase I Trial. Arthritis and Rheumatism. September 1999, Vol. 42, No. 9 SUPPL. page S78, Abstract No. 43.	1-14, 18-19, 25-27, 29-44, 46-47, 51-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11298

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.: 15-17, 20-24, 28, 45, 48-50 and 53-63
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

These claims encompass sequences which were not disclosed in CRF form or a paper copy.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.